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Inhibitory Activity Goblet Depletion and focal inflammatory *Phaleria macrocarpha* Leaves Ethanol Extract on Crypta Mouse after Dextran Sodium Sulphate Induction

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Abstract : Colitis Ulcerative is a major public health problem throught the worldwide. Recently many studies have focused to finding antinflammatory based on the natural product. The study was aimed to investigative the inhibitory activity of *Phaleria macrocarpa* leaves extract on goblet cell in colitis ulcerative. **Methods:** In this study, Swiss mice were induced by 2% dextran sodium sulfate during a week. *Phaleria macrocarpa* leaves extract each dose of 100, 200, and 300 mg daily and aspirin 0.2 mg, administered orally. Histopathological examination of the colon tissue (hematoxylin-eosin staining) was done by counting the number of goblet cells a in five randomly selected fields visual. **The results:** *Phaleria macrocarpa* leaves extract significantly inhibit the depletion of the count of goblet cells (P 0.000) in colitis. *Phaleria macrocarpa* leaves extract significantly reduce the amount of focus of inflammation (P 0.000) in colitis. **Conclusion:** Our results indicated that may have inhibitory activity in colitis through inhibiting reduction in the number of goblet cell.
Keywords : *Phaleria macrocarpa* leaves extract, inflammation, colitis ulcerative, goblet cell depletion, Dextran Sodium Sulfate.

Introduction

The prevalence of chronic inflammatory diseases, especially ulcerative colitis in Asia has increased threefold since the beginning of 1990[1,14]. Based on the results of a retrospective study conducted by Mustika and Triana (2016)[2], it was reported that the number of ulcerative colitis patients at Dr. General Hospital Saiful Anwar (Malang) from 2010 to 2014 reached 8.2%. The prevalence is much higher than the prevalence of ulcerative colitis in Cipto Mangunkusumo Hospital Jakarta from 1991 to 1995 which only reached 2.5% [3]. Because patients with ulcerative colitis continue to increase, the development of anti-inflammatory drugs for ulcerative collective therapy needs attention special.

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The management guide for ulcerative colitis is not yet available. The choice of therapy used for ulcerative colitis is mesalazine, corticosteroids, and immunomodulators. However, all three have unpleasant side effects, such as nausea, vomiting, headaches, hepatitis, and male infertility[4]. In addition, aspirin is also often used for the treatment of ulcerative colitis, but this therapy often results in injury to the gastrointestinal mucosa[5,13]. Therefore, other alternative therapies need to be developed that can minimize the side effects caused.

Recently, there has been a lot of testing of *Phaleria macrocarpa* leaves extract activity related to proof of its efficacy as anti-inflammatory. This is caused by the side effects caused by therapy using steroid and non-steroidal anti-inflammatory drugs[5]. The compounds that are thought to provide anti-inflammatory activity are from phenol compounds[6]. The report of the study conducted by Hendra et al [7]shows that chemical compounds contained in many extracts of the *Phaleria macrocarpha* include kaempferol, myricetin, naringin, quercetin, and gallic acid, where all of these compounds are included in the class of phenolic compounds[8].

The study was aimed to investigate the inhibitory activity of *Phaleria macrocarpa* leaves extract on goblet cell and focal inflammatory in colitis ulcerative.

Materials and Methods

Experimental Animals

Experiments were performed on mature female Swiss mice (body weight range 20-30 g), approximately 20 weeks of age, obtained from Animal Laboratories, National Institute of Health, Research and Development, Ministry of Health (Indonesia). Mice were maintained under controlled in room temperature, humidity, and light (12/12 h light/dark cycle) and allowed ad libitum access to standard mouse chow and tap water. The mice were allowed to acclimate to these conditions for at least 7 days to inclusion in the experiments.

Chemicals and botanical materials

Dextran Sodium Sulfate (molecular weight 500.000 kDa) were purchased from Sigma-Aldrich Chemical Company. All other chemicals of analytical grade were obtained from Merck. .*Phaleria macrocarpa* leaves was obtained from Traditional Medicine Crops Research Institute, Ministry of Agriculture (Indonesia).The material washed in distilled water and allowed to shade dry, the dried material was homogenized in domestic mixture into fine powder, stored in plastic container at room temperature.

Preparation of crude extracts

Crude extract of the respective plant materials was carried out by modified method of Wilson[10].The dried powder of respective plant materials (1000 g) was soaked separately with 3 L of ethanol for 48 hours at room temperature without shaking. The solvent was filter through (Whatman filter paper no.1) & concentrated on rotary vacuum evaporator. Thick extract (16% moisture content) from the leaves of the *Phaleria Macrocarpa.*

Phytochemistry Screening of Plant Extracts

The extract obtained by maceration produced phenol with a grade of 4.4103% or 44.103 GAE/g and Flavonoids with levels of 0.3429% or 3.429 mgQE/g, and have IC_{50} *219.716 µg/mL* (moderate antioxidant intencity)

Study Design And Colitis Administration

The experimental protocols were approved by the Ethics Committee of Faculty of Medicine, Universitas Indonesia (Indonesia). Experimental colon of colities was induced (2% in drinking water) to a pro-inflammatory reagent, dextran sodium sulfate (DSS) for 7 consecutive days. Control animals received 0.9% sterile saline and do not receive an oral exposure of DSS.[11,12,15,17]

Animals were separated into six groups (n=5 per group): control normal, colitis-associated colon carcinogenesis model, aspirin, *Phaleria macrocarpa* leaves extract groups (100, 200, 300 mg/kg body weight).

Animals were randomized and acclimated one week before inducing by DSS. All animals received oral exposure (2% in drinking water) to a pro-inflammatory reagent, dextran sodium sulfate (DSS) for 7 consecutive days. Control animals received 0.9% sterile saline and do not receive an oral exposure of DSS. After induce by DSS, The treatment group received aspirin solution, *Phaleria macrocarpha* extract (100, 200, 300 mg) by oral administration and daily for 2 weeks. In the end of treatment, all animals were sacrificed and the colons were collected.

Histological assessment

Paraffin-embedded gut tissue samples were serially sectioned, and some sections were stained with hematoxylin and eosin. The stained sections were subsequently examined for histopathological changes.

Histological examination of mucosa colon for goblet

Count of goblet cells was quantified by counting the goblet cell number in ten randomly selected visual fields at mucosa colon region under 400x in colon sections from DSS-treated Swiss mice. All slides were analyzed by double investigator who was blinded to the treatment groups. The comparison of each colonic section. Five selected fields shown are representative of all stained sections.

Statistical analyses

All data are expressed as a mean ± standard deviation. Data were analyzed using analyzed of variance (ANOVA) the followed by LSD's test to compare the differences between treatments. Differences were considered statistically significant for $p< 0,05$.

Results

Effect *Phaleria macrocarpa* leaves extract body weight: There was an increase body weight in all groups compared with the beginning of the experiment (Figure 1).

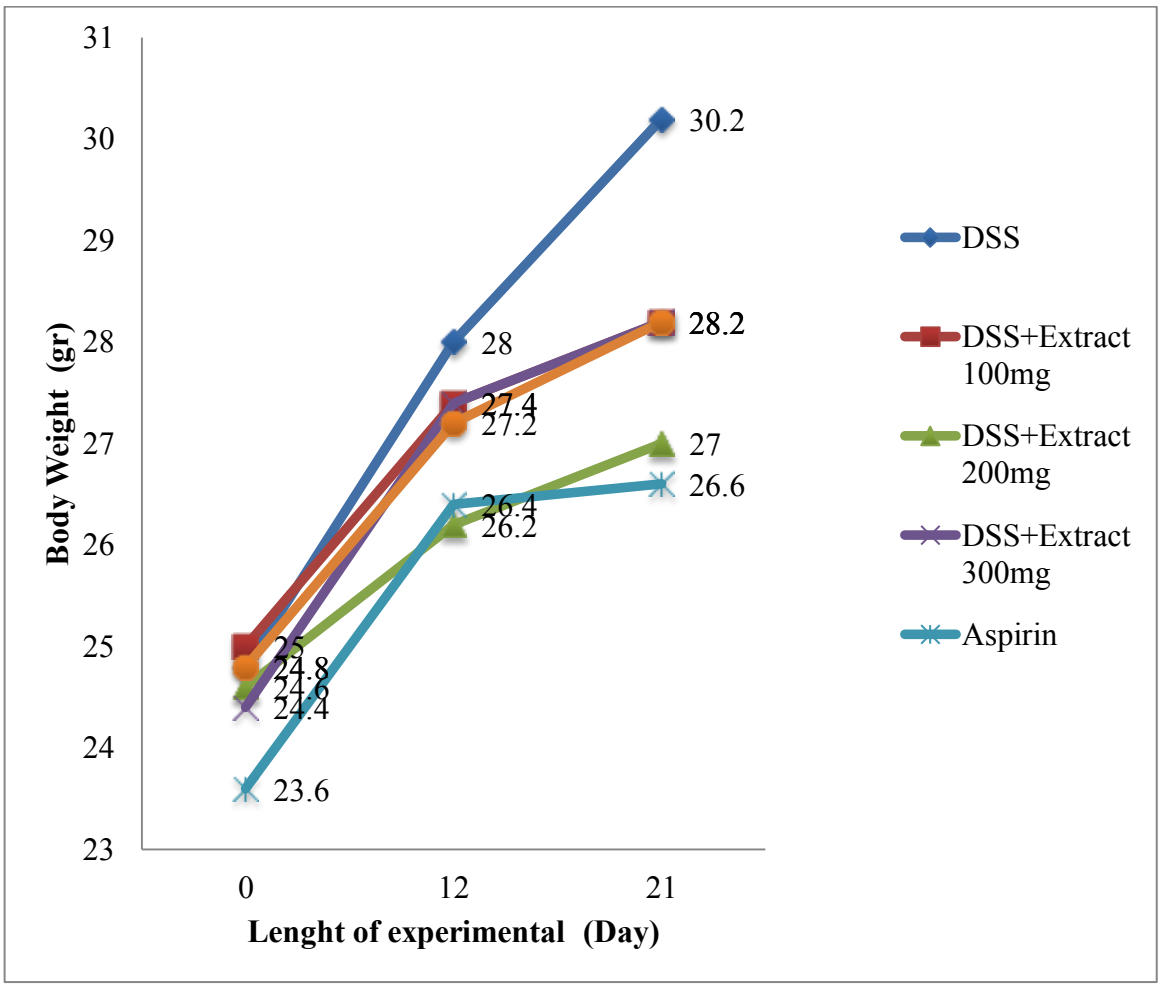

Figure 1. Body weights of mice. Body weights expressed as g, where the interactive effects of duration of experimental (week) and the group were not significant (p>0,05) using Kruskall-Wallis test. Datas are reported as mean ± standard deviation, n=5/group.

Effect of *Phaleria macrocarpa* Leaves extract on goblet cells count

Effect of *Phaleria macrocarpha* leaves extract the histological of goblet cells in mice mucosa colon evaluated by hematoxylin and eosin staining 400 times are shown in Figure 1.

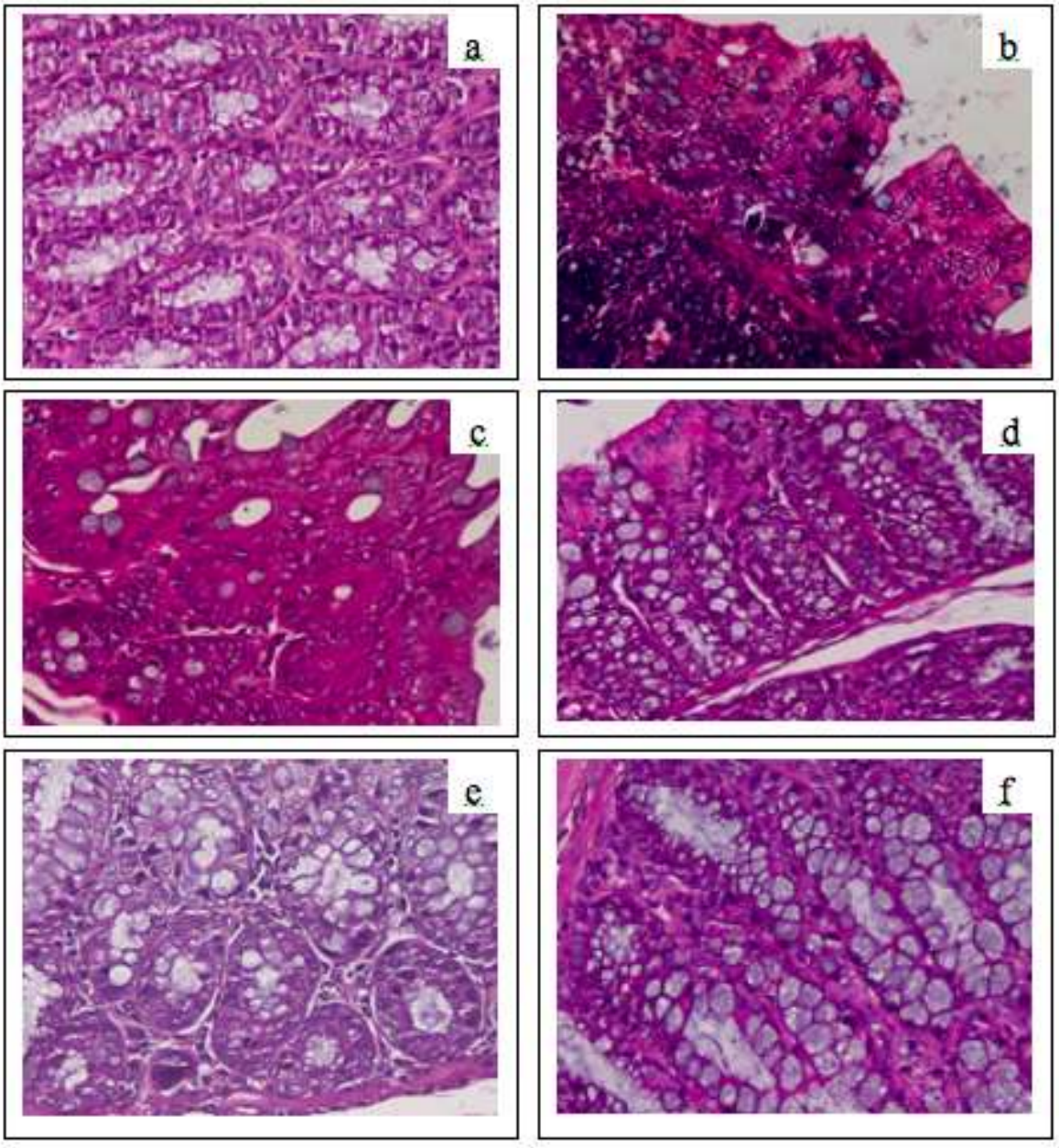

Figure 2. Effect of of *Phaleria macrocarpha* Leaves extract on the histological of goblet cells in mice mucosa colon evaluated by hematoxylin and eosin staining 400 times. (a) DSS, (b) Phaleria macrocarpha leaves extract 100 mg, (c) *Phaleria macrocarpha* leaves extract 200 mg, (d) *Phaleria macrocarpa* leaves extract 300 mg, (e) Aspirin 0.21 mg (f) Normal control.

DSS induced colitis resulted in a significant decrease in count of goblet cell in comparison to control group (P 0.000). After the administration of aspirin and different doses of *Phaleria macrocarpa* leaf extract (100, 200, 300 mg) to colitis ulcerative in mice during 2 weeks a significant increase in count of goblet, respectively (p 0.000) was observed (Table 1).

Table 1. Effect of *Phaleria macrocarpha* leaves extract the goblet cell count after 4 weeks treatment

Treatment (n = 30)	Amount of Goblet Cell	Average Goblet Cell	Sig
DSS	3095	619	0.000
Extract Phaleria Macrocarpha Leaves 100 mg	3973	794.6	
Extract Phaleria Macrocarpha Leaves 200 mg	4873	974.6	
Extract Phaleria Macrocarpha Leaves 300 mg	6612	1322.4	
Aspirin 0.21 mg	4331	866.2	
Control Normal (Without treatment)	6336	1267.2	

Data are expressed mean goblet cells of mice colon tissue in the DSS group, extract of *Phaleria macrocarpa* leaves 100, 200, dan 300 mg, 0.21 mg aspirin, and Control normal (without treatment) showed differences between groups using ANOVA test, n = 5.

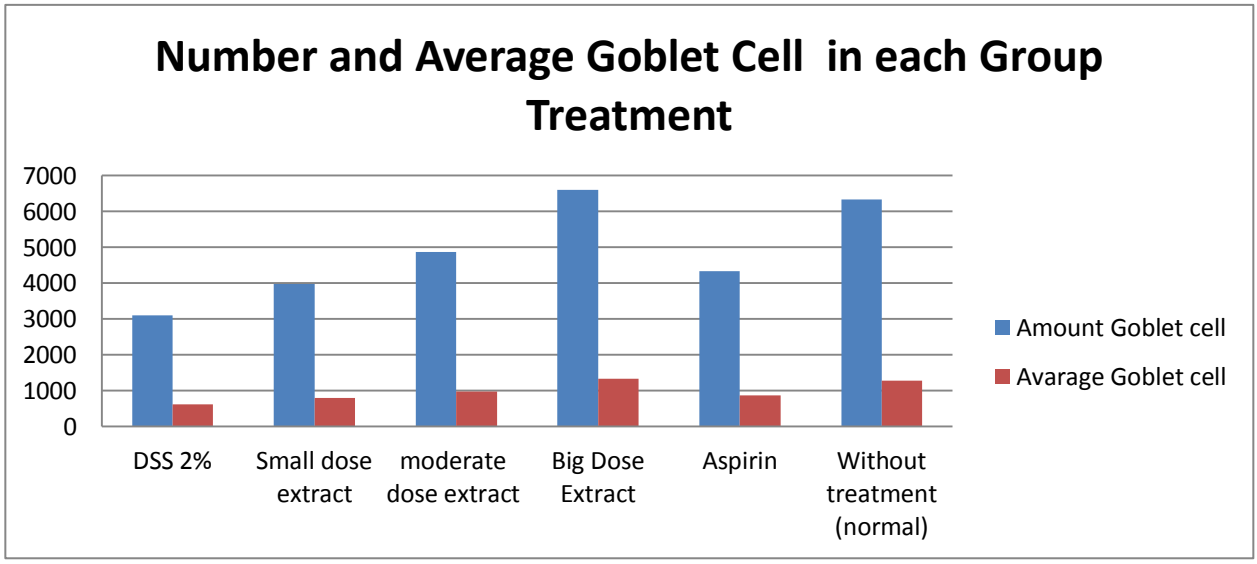

Figure 3. Effect of of *Phaleria macrocarpa* leaves extract the number of goblet cells count each groups, DSS, *Phaleria macrocarpa* leaves extract 100 mg (small dose), (b) *Phaleria macrocarpa* leaves extract 200 mg (moderate dose), *Phaleria macrocarpa* leaves extract 300 mg (high dose), Aspirin 0.21 mg, Normal control.

Effect Effect of *Phaleria macrocarpa* Leaves Extract Focal Inflammatory

Inflammation of colon mucosal tissue from preparations with hematoxylin-eosin staining is seen with 400 times magnification shown in Figure 4, while the number of locations of inflammation is shown in table 2. and the graph of the number of inflammatory locations is shown in Figure 5

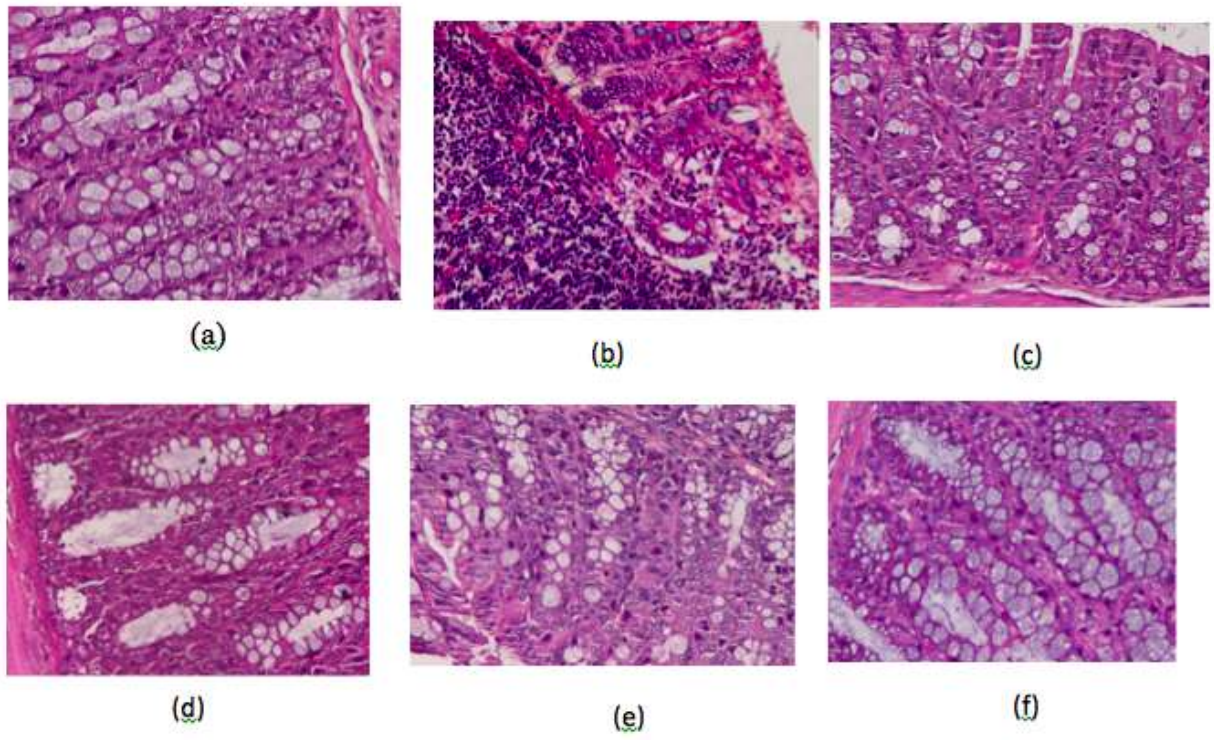

Figure 4. Inflammation of colon tissue in normal control group (a), DSS (b), aspirin (c), *Phaleria macrocarpa* leaves extract 100 mg (e), *Phaleria macrocarpa* leaves extract 200 mg (f) *Phaleria macrocarpha* leaves extract 300 mg, with staining of hematoxylin eosin, 400 times enlargement.

Table 2 . Mean Focal Of Inflammation on Colon Tissue in Normal Mice, DSS, Aspirin, And Treatment with Extract

Treatment (n = 150)	Amount of Inflammation	Average inflammation	Asymp. Sig
DSS 2%	119	23.8	0.000
Small dose extract (100mg)	100	20	
Moderate dose extract (200mg)	78	15.6	
Big dose extract(300mg)	46	9.2	
Aspirin	76	15.2	
without treatment (Normal)	11	2.2	

Table 2. The number of inflammatory sites of mice colon tissue in the normal group, DSS, aspirin, small dose extract, medium dose extract, large dose extract, showed differences between groups using the Kruskall Wallis test, n = 5. It was significant for normal controls to be significant for DSS controls tested using the post hoc test tested using Mann-Whitne.

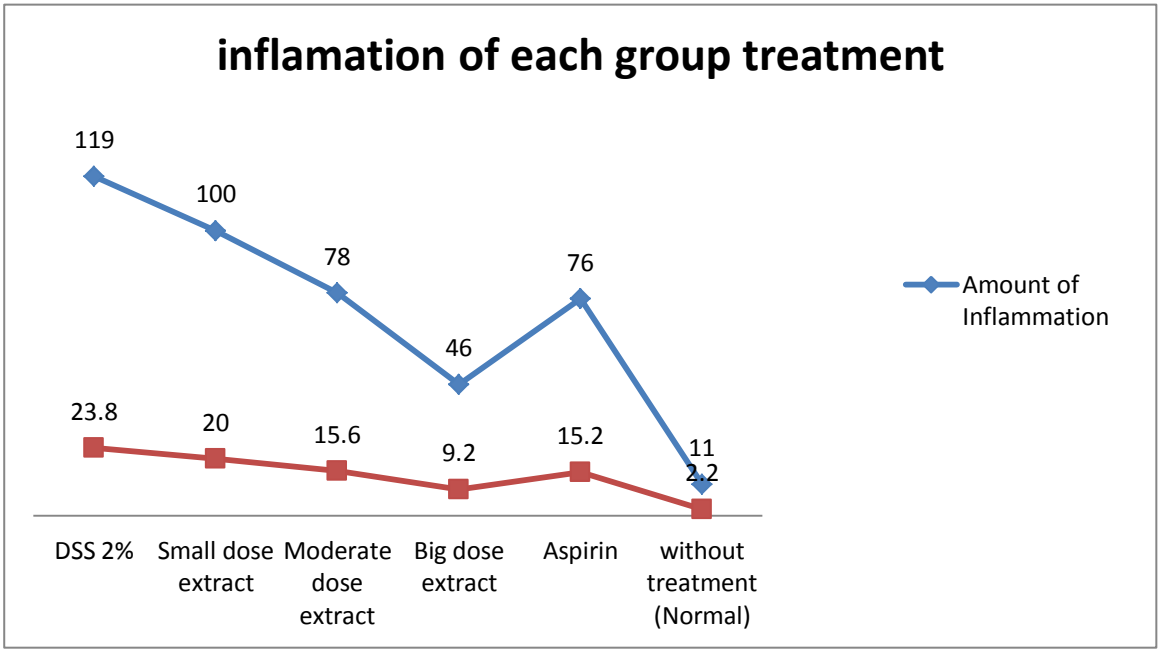

Figure 5. Data are expressed that increasing the focus of inflammation by DSS can be reduced significantly in the administration of aspirin, small dose extract (100 mg) medium (200 mg) and high (300 mg) for 2 weeks.

Discussion

In ulcerative colitis, there are reduced in the number of goblet cells (goblet cell depletion) and thinner colonic mucus layer. Goblet cells are largely responsible for secreting components of the intestinal mucosal barrier and represent a major cellular component of the innate defense. Goblet cell System clearly marked the severity of intestinal inflammation.[16]

The results of the present study indicate that *Phaleria macrocarpa* leaves extract significantly inhibits both the reduction in the number of goblet cell and focal inflammatory in colitis mice model. The extract significantly inhibits the reduction in the number of goblet cell and decrease the number of focal of inflammatory.

In the DSS group there was inflammation with the most amount of focus compared to the treatment group. With the increase in the dose of extracts, the amount of inflammatory focus was also reduced. The administration of high doses of extract (300 mg) the amount of focal inflammation became 9.2 Likewise with the administration of extracts with increased dosage from low to moderate decrease in focus from 20 to 15.6. Our data illustrates that increasing the focus of inflammation by DSS can be reduced significantly in the administration of aspirin, medium dose extract (100 mg) medium (200 mg) and high (300 mg) for 2 weeks.

The data from the study illustrate that in aspirin administration, extracts of low, medium and high doses for 2 weeks can significantly reduce the number of goblet cells caused by DSS. The number of goblet cells in aspirin, extracts of low, medium and high doses for 2 weeks approached normal control.

Currently, there a few reports on experimental colitis. Data reported in this study suggested that *Phaleria macrocarpa* extract investigated for its safety and efficacy and potential clinical applications.

In conclusion, *Phaleria macrocarpa* leaves extract may have inhibitory activity in colitis ulcerative through inhibiting reduction in the number of goblet cell (P 0.000) and decrese focal inflammatory (P 0.000).

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